

# Production of Dryland Barley for Human Food: Quality and Agronomic Performance

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## ABSTRACT

Grain  $\beta$ -glucan content is the most important attribute for barley (*Hordeum vulgare* L.) varieties destined for the human food market. This trait is important because of the blood glucose and cholesterol-reducing properties of  $\beta$ -glucans. High levels of grain protein content, test weight, and seed size and endosperm color may also add value. Seed yield potential, in part, determines the economic feasibility of producing human food varieties. To determine the potential of food barley production in the dryland production areas of the Pacific Northwest of the United States, 33 cultivars and advanced lines reported to vary in  $\beta$ -glucan content were grown in 2006 and 2007 at two locations in northeastern Oregon under dryland cropping conditions. Seed yield, test weight, percentage of plump kernels, grain  $\beta$ -glucan, and grain protein were measured on replicated samples from the four environments, allowing for assessment of average performance as well as genotype  $\times$  environment interaction. Estimates of variance components showed that ~66% of the variability in  $\beta$ -glucan content was attributable to genotype. Cultivars and lines with waxy starch had an average  $\beta$ -glucan value of 55 g kg<sup>-1</sup> compared with 35 g kg<sup>-1</sup> for cultivars and lines with nonwaxy starch. We found significant two- and three-way interactions, but these accounted for much less of the total variation in the measured phenotypes than the main effects of variety, year, and location. Hulless accessions produced an average of 3580 kg grain ha<sup>-1</sup> compared with 4260 kg grain ha<sup>-1</sup> for the hulled accessions. Hulled, waxy-starch varieties appear to have the greatest agronomic potential for dryland production, as they combine high yield potential and grain  $\beta$ -glucan percentage.

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**H**UMAN CONSUMPTION of whole grains helps to control weight gain (Pauline and Rimm, 2003; Arndt, 2006) and reduce the risks of gastrointestinal disorders including cancer (Ranhotra et al., 1991; Department of Health and Human Services and USDA, 2005), coronary and vascular diseases, and type II diabetes (Liu et al., 1999; Anderson et al., 2000; Liu et al., 2000; Pins and Kaur, 2006). In 2006, the U.S. Food and Drug Administration approved a health claim for barley (*Hordeum vulgare* L.), based on the demonstrated reduction in risk of coronary heart disease resulting from consumption of whole-grain barley and barley-containing products (Food and Drug Administration, 2006). To qualify for the health claim, the barley-containing foods must provide at least 0.75 g of soluble fiber per serving. It is the  $\beta$ -glucan fraction of the soluble fiber that is reportedly responsible for lowering low-density lipoprotein and total cholesterol levels (Brown et al., 1999; Kim et al., 2006; Pins and Kaur, 2006).

This new appreciation for the nutritional value of whole-grain barley and barley  $\beta$ -glucan may increase the market for barley products (Bhatti, 1993; Jadhav et al., 1998; Newman and Newman, 2006) and provide new opportunities for  $\beta$ -glucan extraction and enrichment (Knuckles et al., 1992). The traditional markets for barley are malt and animal feed. High  $\beta$ -glucan

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levels are a problem for brewing and, as a consequence, low levels of  $\beta$ -glucan in malt are specified by the malting and brewing industries (Bamforth and Barclay, 1993). Despite evidence that there is variation for feeding quality in barley (Hunt, 1996), a minimum test weight is usually the only quality parameter specified for ruminant feeds. Grain  $\beta$ -glucan is a problem for barley in poultry diets because of “sticky droppings” phenomenon. Solutions to the problem include application of exogenous  $\beta$ -glucanase to grain or overexpression of  $\beta$ -glucanase in transgenics (Almirall et al., 1995; Lisbeth et al., 1996).

$\beta$ -glucan is a nonstarch polysaccharide composed of  $\beta$ -(1 $\rightarrow$ 4)-linked glucose units separated every two to three units by  $\beta$ -(1 $\rightarrow$ 3)-linked glucose and is found only in some grasses and cereals (Carpita, 1996). A health claim for oat (*Avena sativa* L.)  $\beta$ -glucan was issued in 1997 (Food and Drug Administration, 1997). Higher grain  $\beta$ -glucan levels can be achieved with barley than with oats (Aman and Graham, 1987).  $\beta$ -glucan and arabinoxylan are the two major constituents of barley endosperm cell walls, and  $\beta$ -glucan levels are lower in the hull and outer layers of the grain (Henry, 1987).

The genetic analysis of  $\beta$ -glucan has historically been approached with the practical objective of lowering malt  $\beta$ -glucan levels. Because malting involves germination, these analyses involve both  $\beta$ -glucan synthesis and  $\beta$ -glucan degradation by  $\beta$ -glucanases (Han et al., 1995). Three  $\beta$ -glucanases genes are known, but because barley products and  $\beta$ -glucan extraction will likely be based on unmalted grain, these genes will not likely be as important for the development of barley varieties for human nutrition as the genes involved in  $\beta$ -glucan synthesis. Powell et al. (1985) reported that  $\beta$ -glucan content in barley grain is controlled by a simple additive genetic system. Han et al. (1995) reported quantitative trait loci for  $\beta$ -glucan and  $\beta$ -glucanase activity, and their work provided the platform for Burton et al. (2006) to clone and characterize a family of genes responsible for  $\beta$ -glucan biosynthesis. These are members of the family of cellulose synthase-like genes. Although overexpression of these genes in *Arabidopsis* led to  $\beta$ -glucan synthesis, it is not apparent if there is allelic variation at this complex locus in barley germplasm that would allow breeders to select for higher grain  $\beta$ -glucan.

Breeding for high grain  $\beta$ -glucan has been facilitated by the apparent pleiotropic effects of the waxy-starch allele at the granule-bound starch synthase locus (Patron et al., 2002). Positive correlations between grain  $\beta$ -glucan and waxy starch are well documented (Szczodrak et al., 1992; Xue et al., 1997). As a consequence, most barley varieties developed for human nutrition are waxy. Many of the original waxy-starch germplasm accessions were also hulless, leading to the “profile” that high  $\beta$ -glucan food barley varieties are waxy and hulless. Granule-bound starch synthase and the grain nudity (*nud*) locus—which determines hull retention in barley—are

both on chromosome 7H, but they are far enough apart (80 cM) to show independent assortment (Fedak et al., 1972). Hulless varieties may have higher  $\beta$ -glucan due to elimination of the “diluting” effects of the hull (Xue et al., 1997). Hulless types could be more appealing for human food uses since dehulling (pearling) would not be required. There are, however, concerns with hulless varieties due to lower yield potential (Cavallero et al., 2004; Brown, 2005), lower vigor (D. Obert, USDA/ARS, personal communication, 2008), and, in the United States, the lack of a recognized federal grade for hulless barley. This means that hulless barley is not eligible for marketing loans and that hulless barley can only be insured for the same value as feed barley.

There is keen interest in diversifying dryland crop production in the Pacific Northwest of the United States. In 2007, there were 1.55 million ha of dryland cereal production in Oregon, Washington, and Idaho. There are demonstrated advantages to incorporating spring barley into the prevailing wheat (*Triticum aestivum* L.)–fallow cropping system, including suppression of certain diseases and nematodes (Smiley et al., 1994; Young et al., 1994). Data are available on spring malting and feed varieties adapted to the region but not for food barley. To determine the potential for production of human food barley in the region, we assembled an array of spring cultivars and advanced breeding lines and evaluated them for grain  $\beta$ -glucan and other quality and agronomic characters at two representative locations in northeastern Oregon during a two-year period.

## MATERIAL AND METHODS

Four cultivars and 29 advanced breeding lines were obtained from four breeding programs, as described in Table 1. ‘Baronesse’ and ‘Camas’ are hulled, nonwaxy, spring feed cultivars. They are the most widely grown varieties in the growing areas sampled in this study. ‘Salute’ is a commercially available spring variety intended for the human food market. It, and two other spring experimental lines, is hulled and waxy. All the other spring experimental lines but one (‘02WA-7037.9’) are waxy and hulless. This germplasm collection was characterized for food quality traits (grain  $\beta$ -glucan and protein), grain physical quality traits (test weight and kernel plumpness), and grain yield in 2006 and 2007 at the Sherman and Pendleton stations of the Columbia Basin Agricultural Research Center located near Moro and Pendleton, Oregon, respectively. The Moro station is at 45°48' N, 120°73' W. The Pendleton Station is at 45°75' N, 118°63' W. The soil type at both sites is a Walla Walla silt loam (Typic Haploxeroll). Moro and Pendleton are nonirrigated test sites typical of Pacific Northwest dryland cereals production zones. The annual average precipitation at Moro and Pendleton is 280 and 420 mm, respectively; about 75% of the precipitation occurs between 1 October and 30 April. Available moisture and temperature during the two years of testing are shown in Tables 2 and 3. The tests were conducted in the context of longer-term rotations at both locations. At Moro, the preceding component in the rotation was winter wheat in 2006 and fallow in 2007. At Pendleton, the preceding components were spring wheat in

2006 and 2007. More residues from the previous crop were left on the surface at both locations in 2007 to better reflect minimum/no-tillage production conditions.

Moro experiments were planted on 15 March in 2006 and 2007. The Pendleton experiments were planted on 8 March in 2006 and on 13 March in 2007. The experiments were established using a Hege (Hege Equipment, Colwich, KS) small plot drill in 2006 and a Fabro (Fabro, Swift Current, SK, Canada) small plot no-till drill in 2007. The seeding rate was 236 seed m<sup>-2</sup> in all experiments. Agronomic practices, including weed control and fertility management, were conducted in accordance with local practice. The plot size was 1.64 by 6.6 m in 2006 and 2.44 by 9.14 m in 2007; the experimental design was a randomized complete block with four replications. The plots were harvested using a plot combine.

In 2007, there were obvious differences in stand establishment between plots. Vigor ratings were made on 7 May at Moro and 9 May at Pendleton using a 1-to-10 scale, with 10 being a normal stand and 1 a poor stand. To determine if the vigor differences were related to differences in seed viability, the germination percentage of each cultivar–line was determined following the protocol of the Association of Official Seed Analysts (1993). Germination was measured on reserve seed of the same seed lot used for planting in 2007.

For the measurement of grain  $\beta$ -glucan and protein, samples were ground with a Udy Cyclone mill (Udy Corp., Fort Collins, CO) equipped with a 0.5-mm screen. A subsample of this flour was used to determine mixed-linkage  $\beta$ -glucan percentage using the enzymatic assay procedure (EBC Method 3.11.1; Megazyme International Ireland Ltd., Bray, Ireland). Protein concentration was determined, with a subsample of the same flour, by nitrogen combustion (AACC, 2000). A subsample of the flour was oven-dried at 130°C for 1 h to calculate percent moisture.  $\beta$ -glucan and protein content were adjusted for moisture and expressed on a dry-weight basis. The percentage of plump kernels was determined using a 100-g sample and a sieve with 0.24- by 1.9-cm slotted openings per the protocol of the American Society of Brewing Chemists (1992). Grain test weight was calculated based on the weight of a sample of cleaned grain. Grain yield was determined based on the weight of grain harvested from each plot, adjusted for plot size.

Combined analyses of variance were performed across locations and years for all traits using the General Linear Models (GLM) procedure in SAS (SAS Institute, 1999). To better understand environment and year interactions, data from a subset of six representative cultivars–accessions were further analyzed by location and year using GLM. All effects were considered as fixed in the analyses of all data and in the analysis of the subset of lines. The effect of hull type on quality traits was evaluated using orthogonal contrasts. *F*-protected LSD tests were used for mean separation. Simple correlation analysis was performed

using the PROC CORR procedure in SAS. An across-location ANOVA was performed using the 2007 germination and plant vigor data. Contrasts were used to assess the effect of hull type on germination and vigor.

## RESULTS AND DISCUSSION

There were significant differences among cultivars and experimental lines for all traits (Table 4). Mean values for  $\beta$ -glucan varied from 32 to 70 g kg<sup>-1</sup>, with 03AH2214 (a waxy, hullless type) showing the highest percentage and

**Table 1. Barley genotypes tested for  $\beta$ -glucan, protein, and other agronomic traits.**

Cultivar or line	Pedigree	Hull type	Starch type	Source
Camas	ND5976/ND7159	Hulled	Normal	UofI†
01WA-10001.4	Bear/SH97142	Hulless	Waxy	WSU‡
01WA-12501.2	CDC Candle/Meresse	Hulless	Waxy	WSU
01WA-13860.10	SH97142/Merlin	Hulless	Waxy	WSU
01WA-13860-4	SH97142/Merlin	Hulless	Waxy	WSU
01WA-13860-5	SH97142/Merlin	Hulless	Waxy	WSU
02WA-7037.10	WA10314-97/SH97142	Hulless	Waxy	WSU
02WA-7037.25	WA10314-97/SH97142	Hulless	Waxy	WSU
02WA-7037.9	WA10314-97/SH97142	Hulless	Normal	WSU
WA 9892-99	Wanubet/Baronesse	Hulless	Waxy	WSU
Salute (BZ 598-095)	ND-187-636-2/ND-187-631-10//*2 Baronesse	Hulled	Waxy	WestBred, LLC§
Baronesse	Mentor/Minerva//mutant of Vada/4/Carlsberg/Union//Opavsky/Sale/3/Ricardo/5/Oriol/6153 P40	Hulled	Normal	WestBred, LLC
BZ 502-563	Nishino Hoshi/Meresse	Hulled	Waxy	WestBred, LLC
BZ 598-161 (HB 811)	Merlin/waxy Hector	Hulless	Waxy	WestBred, LLC
Meresse-2	Merlin/Baronesse	Hulless	Waxy	WestBred, LLC
YU 501-039 (HB 813)	Merlin/*2 Baronesse	Hulless	Waxy	WestBred, LLC
Yu 599-006	Nebula/Stauwax	Hulled	Waxy	WestBred, LLC
03AH1170	Baronesse/Azhul	Hulless	Waxy	USDA-ARS¶
03AH2214	Azhul/Thuringia	Hulless	Waxy	USDA-ARS
03AH2215	Azhul/Thuringia	Hulless	Waxy	USDA-ARS
03AH2229	Azhul/Thuringia	Hulless	Waxy	USDA-ARS
03AH2616	Bear/Bowman/CDC Alamo	Hulless	Waxy	USDA-ARS
03AH2651	Azhul/CDC Alamo	Hulless	Waxy	USDA-ARS
03AH2689	Azhul/CDC Alamo	Hulless	Waxy	USDA-ARS
03AH2854	Bear/Bowman/CDC Alamo	Hulless	Waxy	USDA-ARS
03AH2873	Bear/Bowman/CDC Alamo	Hulless	Waxy	USDA-ARS
03AH3052	10/Azhul/CDC Alamo	Hulless	Waxy	USDA-ARS
03AH3054	10/Azhul/CDC Alamo	Hulless	Waxy	USDA-ARS
03AH3058	10/Azhul/CDC Alamo	Hulless	Waxy	USDA-ARS
03AH3483	C2-94-220-15-1/Azhul	Hulless	Waxy	USDA-ARS
03AH3491	C2-94-220-15-1/Azhul	Hulless	Waxy	USDA-ARS
03AH6481	CDC Alamo/Otis	Hulless	Waxy	USDA-ARS
03AH6482	CDC Alamo/Otis	Hulless	Waxy	USDA-ARS

†University of Idaho, Moscow.

‡Washington State University, Pullman.

§WestBred, LLC, Bozeman, MT

¶USDA-ARS, National Small Grains Research Facility, Aberdeen, ID.

Table 2. Mean monthly precipitation at Pendleton and Moro, OR, locations in 2006 and 2007.

Month	Monthly precipitation					
	Pendleton			Moro		
	2005–2006	2006–2007	Avg.†	2005–2006	2006–2007	Avg.†
mm						
Sept.	2	19	18	1	1	14
Oct.	35	21	35	46	20	23
Nov.	42	90	53	48	81	43
Dec.	54	59	52	93	64	42
Jan.	88	16	50	68	21	41
Feb.	25	45	39	27	20	29
Mar.	63	42	44	16	17	24
Apr.	72	28	39	46	24	20
May	40	24	38	47	9	21
June	55	30	31	38	14	17
July	3	8	8	2	10	6
Aug.	0	9	12	1	0	7
Total	479	391	419	433	281	287

†Avg. of 77 yr.

Table 3. Mean monthly temperature at Pendleton and Moro, OR, locations in 2006 and 2007.

Month	Monthly temperature					
	Pendleton			Moro		
	2005–2006	2006–2007	Avg.†	2005–2006	2006–2007	Avg.†
°C						
Sept.	14.7	16.4	15.8	15.0	16.1	16.4
Oct.	11.1	9.2	10.0	10.3	10.0	10.6
Nov.	4.2	5.8	4.4	2.8	3.9	4.4
Dec.	−0.3	0.6	1.4	−1.9	−0.8	1.1
Jan.	6.4	−0.6	0.0	3.6	−0.8	−0.3
Feb.	2.2	3.6	2.8	1.4	3.1	2.5
Mar.	6.4	7.8	6.1	4.7	7.0	5.8
Apr.	9.5	8.6	9.5	8.6	7.8	9.2
May	13.3	13.6	13.6	13.1	13.1	13.1
June	17.8	17.8	17.2	17.0	16.4	16.7
July	22.2	23.1	21.1	22.2	22.2	20.9
Aug.	19.5	19.5	20.6	19.5	18.3	20.3

†Avg. of 77 yr.

Camas (a nonwaxy, hulled type) showing the lowest. These values are similar to those reported by Hang et al. (2007) for waxy hulless and standard feed varieties grown in Idaho. Minimum  $\beta$ -glucan levels of  $\geq 50$  g kg<sup>−1</sup> are likely to be required by food barley processors (J. Hamilton, Treasure Valley Renewable Resources, personal communication, 2008). Thus it is apparent that acceptable  $\beta$ -glucan levels should be obtainable under dryland production conditions similar to those at Moro and Pendleton in 2006 and 2007. Mean values for grain protein ranged from 116 g kg<sup>−1</sup> (Camas) to 142 g kg<sup>−1</sup> ('01WA-1000.4'). These values are

Table 4. Means of grain yield, test weight, seed plumpness,  $\beta$ -glucan, and protein of 33 barley genotypes grown at Pendleton and Moro, OR, locations in 2006 and 2007.

Cultivar or line	$\beta$ -glucan content	Protein content	Grain yield	Test weight	Plump kernels
		g kg <sup>−1</sup>	kg ha <sup>−1</sup>	kg hL <sup>−1</sup>	%
Baronesse	35	123	4660	68	63
Camas	32	116	4290	70	70
01WA-10001.4	49	142	3450	76	37
01WA-12501.2	56	130	3710	76	54
01WA-13860-10	53	136	3810	77	60
01WA-13860.4	56	138	3550	75	49
01WA-13860.5	49	137	3830	77	49
02WA-7037.10	41	130	3630	76	51
02WA-7037.25	40	128	3950	75	46
02WA-7037.9	39	129	3930	73	47
WA-9820-98	41	130	3730	75	28
Salute BZ-598-095	51	119	4430	69	80
BZ-502-563	54	124	4600	68	74
BZ 598-161(HB811)	52	130	3710	77	47
Yu 501-0039 (HB813)	55	127	3780	76	54
Yu 599-006	65	125	3700	64	78
Meresse-2	59	138	3270	77	50
03AH1170	50	133	3500	75	57
03AH2214	70	135	3460	77	71
03AH2215	66	141	3460	76	59
03AH2229	62	138	3070	77	78
03AH2616	60	131	3590	77	50
03AH2651	60	135	3480	76	60
03AH2689	62	132	3440	77	63
03AH2854	42	131	3670	75	45
03AH2873	53	131	3650	77	58
03AH3052	64	134	3180	76	42
03AH3054	61	135	3170	76	43
03AH3058	65	139	3200	76	47
03AH3483	58	140	2860	74	57
03AH3491	61	133	3250	75	61
03AH6481	52	128	3960	77	71
03AH6482	52	129	3870	77	72
Mean	53	131	3660	74	57
LSD (0.05)	5.7	8.3	275	1.2	5.4
CV (%)	10.8	6.4	10.9	2.4	13.7

comparable to those reported by Hang et al. (2007) and are fairly typical of grain protein levels for spring barley under dryland conditions. There is genetic variation for grain protein content in barley; in an analysis of 1400 accessions Polan et al. (1968) found a range from 85 to 212 g kg<sup>−1</sup>. The U.S. malting and brewing industry currently specifies a range of protein from 115 to 135 g kg<sup>−1</sup> (American Malting Barley Association, 2008). Although higher grain protein in animal feed and human food should be a desirable attribute, there are currently no premiums paid for high protein, nor are specific protein fractions currently specified.



Mean values for grain yield ranged from 2860 (03AH3483) to 4660 kg ha<sup>-1</sup> (Baronesse). The mean yield for hulled lines was 4270 kg ha<sup>-1</sup> compared to 3580 kg ha<sup>-1</sup> for the hulless lines. This yield reduction associated with the waxy, hulless trait has also been reported in irrigated trials by Brown (2005) and appears to be quite consistent across sites and years. Interestingly, few yield reductions were found when comparing some waxy, hulled cultivars to the feed barley checks. Therefore, cultivars such as Salute and BZ 502-563 may offer the best compromise for dryland growers and processors looking for the waxy trait.

Test weights varied from 64 to 77 kg hL<sup>-1</sup>. Among cultivars, the waxy, hulless lines had significantly higher test weights than the hulled lines. Kernel plumpness ranged from 28 to 80%. In general, the hulled lines had a higher percentage of plump kernels compared to the waxy, hulless lines.

The across-years-and-locations ANOVA revealed that for all traits there were significant three-way interactions except test weight (Table 5). To explore these differences, a subset of six representative cultivars were further examined. The criteria for choosing the subset were as follows: Baronesse and Camas are nonwaxy feed varieties that are currently the most widely grown in the region. Salute is the only commercially available waxy, hulled variety and BZ-502-563 was a high-performing waxy, hulled selection in these trials. Meresse-2 is a commercially available waxy, hulless variety and 03AH6481 was a top performer, of this class, in the trials. Table 6 reports the mean  $\beta$ -glucan, grain protein, grain yield, test weight, and kernel plumpness for each of these cultivars by location and year. In general,  $\beta$ -glucan content was quite stable across locations and years (environments). This large effect of genotype on  $\beta$ -glucan content is also apparent in Table 7. Our results support the assertion that genetics is more important than environment in determining  $\beta$ -glucan content in grain (Gill et al., 1982). However, environment may also influence  $\beta$ -glucan content. Bendelow (1975) reported that dry conditions before harvest increase  $\beta$ -glucan, whereas Savin et al. (1997) and Savin and Nicolas (1996) reported a decrease in  $\beta$ -glucan due to moisture stress during grain filling. In our study, the  $\beta$ -glucan content of Salute varied from 41 to 58 g kg<sup>-1</sup> depending on environment. If  $\geq 50$  g kg<sup>-1</sup>  $\beta$ -glucan content is required by processors, then growing Salute under low-rainfall dryland conditions, such as those found in Moro, may not meet specifications. Additional research on the main and interaction effects of moisture availability and nitrogen fertility on grain  $\beta$ -glucan is necessary.

Genotype had a lesser effect on grain protein: the location effect accounted for 57% of the total sums of squares. Average proteins were 14.5 and 11.8% for Pendleton and

**Table 5. Estimates of variance components for traits measured in 33 barley genotypes for seed yield, test weight, seed plumpness,  $\beta$ -glucan, and protein grown at Pendleton and Moro, OR, locations in 2006 and 2007.**

Component of variance†	$\beta$ -glucan content	Protein content	Seed yield	Test weight	Plump kernels
$\hat{\sigma}_G^2$	7.336**	3.024**	2,729,384**	178.500**	2,586.088**
$\hat{\sigma}_L^2$	1.881*	471.469**	120,075,064**	24.613*	17,057.956**
$\hat{\sigma}_Y^2$	9.435**	0.021ns‡	281,959,039**	184.363**	40,500.285**
$\hat{\sigma}_{R(YL)}^2$	4.711**	8.652**	889,439**	25.483**	1,061.061**
$\hat{\sigma}_{GY}^2$	0.446ns	1.239*	487,931**	20.047**	269.127**
$\hat{\sigma}_{GL}^2$	0.374ns	0.993ns	240,716*	4.422ns	281.348**
$\hat{\sigma}_{YL}^2$	3.258*	2.927*	1,548,798**	9.818ns	6,931.726**
$\hat{\sigma}_{GYL}^2$	0.616*	1.189*	386,254**	4.767ns	155.974**

\*Significant at  $P < 0.05$ .

\*\*Significant at  $P < 0.01$ .

†G, genotype; L, location; R, replicate; Y, year.

‡ns, not significant.

Moro, respectively. The environment is known to have significant effects on grain protein, with moisture and available nitrogen having major effects (Fathi et al., 1997). Torp et al. (1981), for example, reported that the protein content of barley of the same genotype varied from 8.1 and 14.7% at different locations with similar nitrogen fertilization levels.

Genotype  $\times$  environment interaction for grain yield and yield components is the subject of extensive research in crops (Manjit and Gauch, 1996) and in barley (van Oosterom et al., 1993). In our experiments, the individual main effects of genotype, location, and year were principal determinants for test weight, percentage of plump kernels, and grain yield (Tables 5 and 7). These results can be attributed to the preponderance of lower-yielding hulless lines vs. a limited number of higher-yielding hulled types.

There are two general reasons why hulless accessions may yield less than hulled types. The first is simply the weight of hulls, which estimated to be 11 to 13% of the average grain yield (Bhatty et al., 1975). Second, there is a much shorter history of breeding for agronomic performance in hulless than for hulled types in the U.S. Pacific Northwest. Considerable improvement can be expected. In western Canada, for example, a concerted effort at improving yield in hulless types was initiated in 1970 (Bhatty, 1993).

In our experiments, the yield of hulless lines was significantly lower the second year due to poor stand establishment. At the time of seeding in the second year of the experiment, there was more residue on the surface of the soil at both locations to better represent the minimum-no-tillage production systems of the Pacific Northwest.

Table 6. Means of grain yield, test weight, seed plumpness,  $\beta$ -glucan, and protein of six representative barley genotypes grown at Pendleton and Moro, OR, locations in 2006 and 2007.

Cultivar or line	$\beta$ -glucan content	Protein content	Grain yield	Test weight	Plump kernels
	g kg <sup>-1</sup>		kg ha <sup>-1</sup>	kg hL <sup>-1</sup>	%
2007 Pendleton					
Baronesse	33	148	4940	68.0	78.3
Camas	33	143	4140	70.8	83.3
Salute BZ-598-095	58	141	4410	68.5	87.1
BZ-502-563	54	147	4520	66.3	79.8
Meresse-2	59	147	2290	75.8	74.4
03AH6481	57	147	3340	74.5	81.5
Mean	49	145	3941	70.6	80.7
LSD (0.05)	5	23	528	2.3	7.1
CV%	3.6	6.2	8.9	2.1	5.8
2006 Pendleton					
Baronesse	35	130	5580	68.5	66.2
Camas	34	131	5700	70.5	64.1
Salute BZ-598-095	54	123	5680	71.0	86.7
BZ-502-563	53	130	5800	67.8	78.4
Meresse-2	57	148	4720	78.5	49.0
03AH6481	56	145	5250	77.5	64.1
Mean	48	134	5455	72.3	68.1
LSD (0.05)	4	1	450	1.9	7.9
CV%	3.5	2.8	5.5	1.7	7.7
2007 Moro					
Baronesse	35	117	3740	67.8	72.3
Camas	28	95	2790	68.8	74.3
Salute BZ-598-095	41	99	3160	68.5	86.6
BZ-502-563	58	97	3420	68.8	92.0
Meresse-2	64	141	2080	75.8	53.5
03AH6481	51	107	3390	74.5	80.0
Mean	46	109	3097	70.7	76.4
LSD (0.05)	8	27	785	2.2	12.6
CV%	6.5	9.6	16.8	2.0	10.9
2006 Moro					
Baronesse	34	97	4360	65.8	38.5
Camas	32	96	4530	70.5	59.3
Salute BZ-598-095	50	115	4460	67.3	63.1
BZ-502-563	50	123	4660	69.3	46.7
Meresse-2	58	118	3980	79.8	23.7
03AH6481	45	113	3840	78.3	61.5
Mean	45	110	4305	71.8	48.8
LSD (0.05)	7	6	516	5.6	14.0
CV%	5.7	2.0	8.0	5.1	19.1

Visible differences in stand establishment were observed approximately 1 mo after seeding at both locations. These differences were assessed using a subjective visual rating for vigor. There was a significant difference ( $P < 0.001$ ) for the contrast of hulless vs. hulled for vigor score (data not shown). The mean vigor rating values ranged from 2

Table 7. Contribution of factors to total variation obtained in the analysis of variance for traits measured in 33 barley genotypes for seed yield, test weight, seed plumpness,  $\beta$ -glucan, and protein grown at Pendleton and Moro, OR, locations in 2006 and 2007.

Source	Contribution of factor (%) <sup>†</sup>				
	$\beta$ -glucan content	Protein content	Seed yield	Test weight	Plump kernels
Genotype (G)	65.7	11.7	14.6	67.68	40.1
Replicate	5.3	4.18	1.78	1.63	2.2
Environment (E)	0.5	57	20.04	0.32	8.3
Year (Y)	2.6	0.003	47	2	19.6
G $\times$ E	3.4	3.5	1.28	1.6	4.4
G $\times$ Y	4.0	0.4	2.6	0.2	4.2
G $\times$ E $\times$ Y	6.4	4.8	2.32	2.0	5.7

<sup>†</sup>Contribution of each factor defined as (factor sum squares/total sum squares  $\times$  100).

Table 8. Pearson's simple correlation coefficients and  $P$  values for traits measured in 33 barley genotypes grown at Pendleton and Moro, OR, locations in 2006 and 2007.

Trait	Trait			
	Test weight	Plump kernels	$\beta$ -glucan content	Protein content
Seed yield				
Pearson's correlation coef.	-0.08	-0.36	0.20	0.07
$P$ value	0.12	<0.0001	<0.0001	0.19
Sample size, no.	480	480	240	240
Test weight				
Pearson's correlation coef.		-0.18	0.01	0.01
$P$ value		0.00	0.89	0.77
Sample size, no.		480	240	240
Plump kernels				
Pearson's correlation coef.			-0.10	-0.10
$P$ value			0.0	0.3
Sample size, no.			240	240
$\beta$ -glucan				
Pearson's correlation coef.				0.95
$P$ value				<0.0001
Sample size, no.				240

to 10 at Pendleton and from 1 to 10 at Moro. The mean vigor rating was 9.8 for hulled cultivars and lines at both Pendleton and Moro, while the mean vigor rating for the hulless lines was 6.5 at Pendleton and 4.4 at Moro. Hulless type may be more prone to physical damage to the embryo during harvest, leading to lower germination rates. However, in our experiments, differences in stand establishment were not due to seed viability. Germination percentages on reserve seed from the same lots used for seeding the 2007 trials were all >96%. However, hulless varieties may suffer embryo damage that is not sufficient to reduce germination but is sufficient to reduce vigor.

Additional research is warranted on the causes of poor stand establishment of hulless barley under stressful conditions. Potential causes of differences in stand establishment under reduced tillage include response to soil pathogens (Bockus and Shroyer, 1998) and response to lower temperatures during germination (Franzluebbers et al., 1995). It is important to understand the basis of poor stand establishment because the hulless types had, on average, significantly higher ( $P < 0.001$ ) grain  $\beta$ -glucan and grain protein. These significant differences, however, are due in part to the confounding effects of waxy vs. nonwaxy starch and hulled vs. hulless in this sample of germplasm. All but one of the hulless lines were waxy and would be expected to have higher  $\beta$ -glucan due to the pleiotropic effects of the waxy allele and hulless alleles on  $\beta$ -glucan content (Xue et al., 1991, 1997). Three of the five hulled accessions have waxy starch (Salute, YU 599-006, BZ 502-563). The average  $\beta$ -glucan level of these three accessions is significantly higher than those of the nonwaxy, hulled checks ( $P < 0.001$ ) and not significantly different from the average of the hulled, waxy types.

$\beta$ -glucan showed a modest positive correlation with grain yield (Table 8). Hang et al. (2007) found a modest negative correlation ( $r = -0.11$ ) of  $\beta$ -glucan with grain yield, and they concluded that this could complicate simultaneous improvement for both traits. Although statistically significant, correlations of this size (either negative or positive) may be of little biological importance. Their germplasm collection did not include high-yielding and high  $\beta$ -glucan varieties, such as Salute. We found a very high and positive correlation between grain protein and  $\beta$ -glucan. Fastnaught et al. (1996) and Hang et al. (2007) also reported positive relationships between the two traits.

Although the current market is most interested in  $\beta$ -glucan, there are potential markets for grain protein, making this positive correlation of  $\beta$ -glucan with protein of greater potential economic value. Additional research, focusing on a set of high  $\beta$ -glucan cultivars and/or breeding lines, is needed to determine if this positive association would be useful for indirect selection; grain protein is simpler and less expensive to measure than grain  $\beta$ -glucan.

## CONCLUSIONS

The approval of the barley health claim by the U.S. Food and Drug Administration, coupled with the increasing health consciousness of an American public faced with alarming rates of obesity and coronary heart disease, may increase interest in, and markets for, food barley. To meet this demand, barley processors are likely to require production of waxy barley cultivars due to their high  $\beta$ -glucan contents. However, our research indicates that there are significant production problems and yield reductions currently associated with the hulless, waxy trait. Therefore, waxy, hulled lines such as Salute and BZ 502-563 are

likely the best alternative for dryland growers and processors. Mechanical removal of the hull is easily accomplished by pearling. Because  $\beta$ -glucan levels are low in the hull and outer layers of the seed coat (Henry, 1987), little value would be lost in this operation. Additionally, both Salute and BZ 502-563 show no production or yield differences when compared to Baroness and Camas, the hulled, non-waxy lines that are widely grown in the dryland production zones of the Pacific Northwest. Thus, spring cultivars currently exist that are agronomically competitive with current feed barleys and have high  $\beta$ -glucan that could support a niche food barley market in the Pacific Northwest. Development of winter habit food barleys would provide growers and industry with even more productive choices.

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